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REVIEW ARTICLE

A retrospective analysis of toxicity studies in dogs and impact on the chronic reference dose for conventional pesticide chemicals

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Abstract
Prior to October 2007, the US Environmental Protection Agency (EPA) required both 13-week and 1-year studies in Beagle dogs be submitted in support of registration for pesticides. Following an extensive retrospective analysis, we (the authors) determined that the 1-year toxicity dog study should be eliminated as a requirement for pesticide registration. The present work presents this retrospective analysis of results from 13-week and 1-year dog studies for 110 conventional pesticide chemicals, representing more than 50 classes of pesticides. The data were evaluated to determine if the 13-week dog study, in addition to the long-term studies in two rodent species (mice and rats), were sufficient for the identification of no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) for the derivation of chronic reference doses (RfD). Only pesticides with adequate 13-week and 1-year duration studies were included in the present evaluation. Toxicity endpoints and dose-response data from 13-week and 1-year studies were compared. The analysis showed that 70 of the 110 pesticides had similar critical effects regardless of duration and had NOAELs and LOAELs within a difference of 1.5-fold of each other. For the remaining 40 pesticides, 31 had lower NOAELs and LOAELs in the 1-year study, primarily due to dose selection and spacing. In only 2% of the cases were additional toxic effects identified in the 1-year study that were not observed in the 13-week study and/or in the rodent studies. In 8% of the cases, the 1-year dog had a lower NOAEL and/or LOAEL than the 13-week study, but there would have been no regulatory impact if the 1-year dog study had not been performed because adequate data were available from the other required studies. A dog toxicity study beyond 13-weeks does not have significant impact on the derivation of a chronic RfD for pesticide risk assessment.

Keywords: *Animal testing; dog toxicity studies; health-based reference values; pesticide chemicals; retrospective analysis*

Contents

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Introduction

The current data requirements for pesticide registration typically include an extensive number of laboratory animal toxicity studies. These include oral, dermal, and inhalation toxicity studies in mice, rats, and dogs of various durations (and exposure conditions). These studies are used to identify lowest observed adverse effect levels (LOAELs) and no observed adverse effect levels (NOAELs) of critical toxicities, which are used for hazard assessment and to set regulatory values such as reference doses (RfDs). The RfD is defined as an estimate, within an order of magnitude, of the exposure level assumed to be without appreciable risk of adverse health effects and is based on identifying a NOAEL for a critical effect determined from laboratory animal studies (US EPA, 2002; EPA/630/P-02/002F). The entire toxicity database is used to characterize target organ toxicity, potential carcinogenicity, sex and age differences in toxicity response, and, when possible, the mode of toxic action. To minimize the number of animals required for pesticide registration while adequately describing the potential for hazard, it is important, in regulatory toxicology, to ensure that animal studies are scientifically appropriate and necessary. The need for multiple toxicity studies using dogs has long been a subject of debate.

There is currently no consistent international standard in regulatory guidelines specifying the appropriate duration for dog studies. The US Environmental Protection Agency (US EPA) had for some time required a nonrodent subchronic and chronic study be submitted to support a pesticide registration for food use conventional chemical likely to result in repeated exposure over a significant time. In October 2007, however, the US EPA dropped the 1-year dog study requirement while retaining the requirement for the 13-week dog toxicity study for conventional pesticide chemicals, as discussed later. Canada requires both a 13-week and 1-year dog study for pesticide registration. For chronic toxicity assessment, Japan requires at least 1 study in a nonrodent species. Although there may be many choices for which species to use, it is common practice for the nonrodent species to be the dog. The European Union (EU) always requires a 13-week dog study but if the dog is clearly the most sensitive species or the best model for humans (e.g., rodent-specific effects can be demonstrated), a 1-year study would be triggered.

There have been a number of separate efforts to analyze the results of dog toxicity studies to determine their impact in risk assessment. Gerbracht and Spielmann (1998) noted no significant differences in species-specific organ toxicities among rats, mice, and dogs in 13-week and 52- or 104-week studies. However, hemotoxic effects were more often detected in dogs. Doe et al. (2006) evaluated data on NOAELs, LOAELs, and critical toxicities on 28 pesticides provided by the US EPA's Office of Pesticide Programs (OPP). These authors concluded that "the rat and the dog can respond with a differential sensitivity, i.e., the same effects occur but at different dose levels, and less commonly with a different susceptibility, i.e., different effects occur, to the same chemical, and both should be retained as test species for evaluating the systemic toxicity of agricultural chemicals." Of the chronic RfDs in the US EPA's pesticide database, it was reported that 38% were based on data from a dog study (US EPA, 2005a). Therefore, the use of the dog as a second species that is phylogenetically removed from the rat appears to be important to retain for the purpose of evaluating the systemic toxicity of pesticide chemicals.

In 2001, Spielmann and Gerbracht (also Box and Spielmann, 2005) published a comprehensive analysis of data from dog studies on 172 pesticides that had been submitted to the Federal Institute of Health Protection of Consumers and Veterinary Medicine (Germany). The focus of this analysis was on whether dog studies >13 weeks provided important additional information not provided by studies of shorter duration. They reported that "analysis of the severity of organ-specific toxic effects of pesticides revealed that chronic long-term studies (52/104 weeks) in dogs do not provide specific additional information to 26-week studies in the same species." They further stated that "safety testing of pesticides in dogs should be limited to subchronic (13-week) studies since an extension of the duration of the studies does not provide additional essential information." The recommendation for a study of 13-week duration was supported by the finding that in only 5% was new and relevant information on the toxic properties of the pesticide provided by chronic dog studies that was not seen in dog studies of shorter duration or in studies with rats or mice. The authors concluded, "Chronic studies are only of limited value since they added essential information to that obtained in subchronic studies only in about 5 percent of the cases" (Box and Spielmann, 2005).

In March 2005, EPA issued a notice in the Federal Register to revise the Part 158 toxicology data requirements supporting conventional pesticide registration (March 11, 2005, 70 FR 12275). In the preamble of that notice, based on a retrospective analysis of a large body of 90-day and 1-year dog studies in its database, EPA proposed to eliminate the 1-year dog toxicity (but retain the 90-day dog toxicity study) data requirement for conventional pesticides chemicals. EPA solicited review and comment by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) on the results of the preliminary retrospective analysis of data from dog toxicity studies for reference dose (RfD) derivation (US EPA, 2005a).

The SAP (May 5–6, 2005) reviewed the Agency's analysis of the findings from different duration dog toxicity studies for conventional pesticide chemicals (US EPA, 2005b). The Panel made a number of recommendations and encouraged the Agency to continue their analyses of the dog toxicity studies with a larger database and also specified that "if the results of the analysis continue to indicate little added value from the one-year dog studies, the Agency could move toward eliminating them on a stronger basis." Following the SAP review, the EPA addressed the SAP comments by conducting a larger retrospective analysis of results from 13-week and 1-year dog studies for pesticide compounds (US EPA, 2006). As a result of this analysis, the US EPA Office of Pesticide Programs eliminated the routine requirement of the 1-year dog toxicity while retaining the 13-week study requirement (Federal Register Notice, 2007). The purpose of this paper is to present the US EPA 2006 analysis.

Methods

The retrospective analysis began with a review of chronic RfDs established through December 2005 by the US EPA for 330 conventional pesticide chemicals. EPA's Office of Pesticide Programs (OPP) establishes chronic reference doses (RfD) for conducting dietary risk assessment in support of pesticide registration and re-registration. OPP toxicology databases were considered to determine whether the available data were sufficient to compare the results of the 1-year and 13-week studies in the dog without regard to the study/species used as the basis for the chronic RfD.

The final selection criterion for including pesticides in this retrospective analysis was the availability of adequate studies in the dog with a duration of ≥1 year and with a duration of 13 weeks. Study adequacy is defined by the conduct of the study meeting all Subdivision F guideline requirements (OPPTS 870.4100 and OPPTS 870.3150) and toxicology Data Evaluation Reports (DERs) prepared by EPA scientists for the studies containing sufficient detail for critical comparison of study results. The DER is the official record of independent review that contains conclusions for a submitted study and includes data review and analysis of the study results, including survival, body weight, clinical chemistry, hematology, urinalysis, organ weights, and gross and histopathology findings. New pesticides, not yet registered, or pesticides that have had their registrations cancelled were not included in this analysis.

After considering the study adequacy of each pesticide candidate, a total of 110 pesticides were identified for inclusion in this retrospective analysis. The pesticides included in this updated retrospective analysis represent more than 50 chemical classes, e.g., organophosphates, carbamates, pyrethroids, triazoles, sulfonylureas, etc. (US EPA, 2006). Of the 110 pesticides, 55 had a chronic RfD based on a dog study and 55 pesticides had a chronic RfD based on a rodent study. Additional analyses were conducted on the dose-response data for pesticides that showed differences in NOAELs or LOAELs of 1.5-fold or greater between the results of the 13-week and 1-year dog studies. This analysis included a more critical examination of the incidence, severity, and magnitude of the effects in the target organ at each dose level in the studies as well as the doses selected and the overall experimental design of each study.

When a pesticide had differences in NOAELs and LOAELs of 1.5-fold or greater between the results of the 13-week and 1-year dog studies and the difference could not be clearly ascribed to dose spacing selection or experimental variations, a further review of the impact of the absence of the chronic dog study on the overall risk assessment for the pesticide was conducted. To do this, the entire toxicology database for the pesticide was reevaluated from this perspective (i.e., an assumption of no 1-year dog study). This often entailed re-selection of a chronic RfD and resulting recalculation of the chronic dietary risk for the most sensitive population subgroup (US EPA, 2000).

Results

The detailed information regarding the results of this retrospective analysis can be found in Tables 2A and 3A in the document located at http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064807ddb6c. Figure 1 of this paper provides an overall summary of the comparative analysis of the NOAELs/LOAELS from 13-week and 1-year dog toxicity studies for conventional pesticide chemicals; and Table 1 lists the conventional pesticide chemicals included in the analysis.

When the results of the 13-week and 1-year dog studies were compared for 110 pesticides, the degree of correlation in the data for endpoints from two independently conducted studies with different durations is quite remarkable. As shown in Figure 1, 70 pesticides (listed in Table 1A) out of 110 (~64%) generally showed similar target organ toxicities and had NOAELs and LOAELs with differences less than 1.5-fold.

Of the remaining 40 pesticides that did show differences in NOAELs and/or LOAELs of 1.5-fold or greater between the two studies, an additional analysis was conducted on the dose-response data. An explanation of the differences seen in the two duration dog studies for 31 (listed in Table 1B) of the 40 pesticides follows.

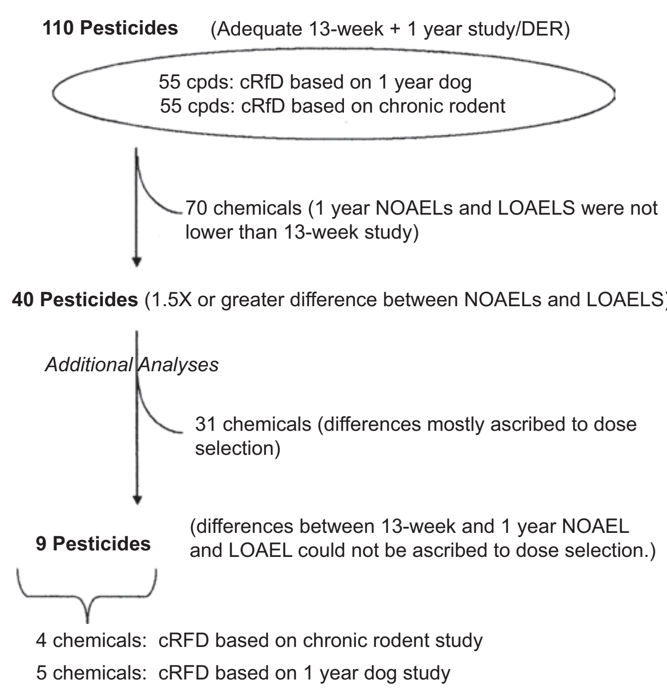

Figure 1. Comparison of NOAELs and LOAELs from 13-week and 1-year dog studies.

Table 1. List of conventional pesticide chemicals evaluated.

A. NOAELs/LOAELs in 1-Year Study Not Lower Than 13-Week Study (70 Chemicals)
Acetamiprid
Amitraz
Benfluralin
Bensulide
Bifenazate
Bromoxynil phenol
Bromuconazole
Butafenacil
Carfentrozone-ethyl
Chlorethoxyfos
Chlorfenopyr
Clodinafop
Cloquintocet-mexyl
Cyazifamid
Cyfluthrin
Cyhalofop butyl
Cymoxanil
Deltamethrin
Desmidipham
Dicloran
Diflubenzuron
Dimethomorph
Emamectin
Famoxadone
Fenpropathrin
Fenpyroximate
Fipronil
Flonicamid
Flucarbazone
Fludioxanil
Flumioxazin
Formosulfuron
Glufosinate ammonium
Imazethapyr
Indoxacarb
Iodosulfuran
Isoxadifen ethyl
Mepanipyrim
Mepiquat chloride
Methoxy-fenozide
Methyl Parathion
Metolochlor
Paraquat
Phorate
Phostebupirim
Pinoxaden
Pirimisulfuron methyl
Prallethrin
Prohexadione calcium
Propiconazole
Propochlor
Pymetrozine
Pyraflufen-ethyl
Pyridate
Pyriproxyfen
Rimsulfuron
Simazine
Sulfentrazone
Sulfosate
Sulfosulfuron
Teflubenzuron
Tepraloxydim
Thiabendazole
Thiacloprid
Tralkoxydim
Triadimefon
Triadimenol
Trifloxystrobin
Triflusulfuron-methyl
Zoxamide
B. Differences in 1-Year and 13-Week Dog Studies Attributed to Dose Selection and/or Spacing (31 Chemicals)
Acibenzolar-*S*-methyl
Azoxystrobin
Boscalid
Cadusafos
Clethodim
Cyprodinil
Dicofol
Diflufenzopyr
Dinotefuran
Epoxiconazole
Ethylene thiourea
Etoxazole
Fenhexamid
Fluazifop-butyl
Flufenpyr-ethyl
Fluoxastrobin
Fosetyl Al
Hexaconazole
Mefenpyr-diethyl
Mesosulfuron methyl
Myclobutanil
Penoxulam
Prosulfuron
Pyrimethanil
Spinosad
Spirodiclofen
Spiroxamine
Tebufenozide
Thiophanate methyl
Triazamate
Trifloxysulfuron-sodium
C. Differences in 1-Year and 13-Week Dog Studies NOT Attributed to Dose Selection and/or Spacing (9 Chemicals)
Bifenthrin
Bispyibac sodium
Cypermethrin
Fluazinam
Hexazinone
Mancozeb
Tebuconazole
Thiamethoxam
Thiram

For 10 pesticides—Acibenzolar-s-methyl, Azoxystrobin, Clethodim, Cyprodinil, Diflufenzopyr, Etoxazole, Fenhexamid, Flufenpyr-ethyl, Mefenpyr-diethyl, and Prosulfuron—only the NOAELs had a 1.5-fold or greater difference but the LOAELs were similar and showed similar toxicities. These differences could be attributed to dose selection and dose spacing.

For 10 pesticides—Boscalid, Dicofol, Fluoxystrobin, Hexaconazole, Myclobutanil, Penoxulam, Spinosad, Spiroxamine, Tebufenozide, and Trifloxysulfuron-sodium—the 13-week study would have adequately characterized the toxicity and identified a protective NOAEL.

For three pesticides—Cadusafos, Thiophanate-methyl, and Triazamate—a NOAEL was not established in the 13-week study and therefore an extrapolated NOAEL (10× default factor for lack of a study NOAEL) was used in the analysis. In these three cases, the extrapolated NOAEL was protective of the effects seen in the chronic study. It was also noted that Cadusafos is a cholinesterase-inhibiting organophosphate pesticide that generally reaches steady state cholinesterase inhibition by approximately 30 days; the lower LOAEL (0.005 mg/kg body weight [bw]) in the 1-year dog study was due to dose selection.

For eight pesticides—Ethylene thiourea, Dinotefuran, Epoxiconazole, Fluazifop-butyl, Fosetyl aluminum, Mesosulfuronmethyl, Pyrimethanil, and Spirodiclofen—reasons for the differences in NOAELs and/or LOAELs of 1.5-fold or greater between the two studies are explained for each case as follows:

- Ethylene thiourea—the primary toxic effect for this chemical is perturbation of thyroid homeostasis. Effects on thyroid hormones should be detectable within several weeks. Thus, the approximate 3-fold difference seen in NOAELs and LOAELs was ascribed to dose selection and experimental variability.
- Dinotefuran—In the 1-year study with Dinotefuran, the LOAEL was based on thymus weight changes that were not dose related, not statistically significant and were largely driven by one dog in the control group with a thymus weight about 2-fold heavier than the other controls. Therefore, the 1-year NOAEL and LOAEL would be 20 mg/kg/day and 108 mg/kg/day, respectively, based on decreased body weight and body weight gain. Considering these results, Dinotefuran would not have required further analysis because there would no longer be differences in NOAELs and/or LOAELs of 1.5-fold or greater between the two studies.
- Epoxiconazole—In the 1-year study with Epoxiconazole, hematologic effects were seen at 1.5 mg/kg/day in males, which was the same dose level as the NOAEL in females in this study. However, the magnitude of changes for hematologic effects in males treated with 1.5 mg/kg/day were similar among all treatment groups. Therefore, 1.5 mg/kg/day should be selected as the NOAEL for the 1-year dog study and LOAEL would be 14.4 mg/kg based on liver effects. With this reevaluation, the effects are similar and the small differences in LOAELs between the 13-week and 1-year dog studies are due to dose spacing.
- Fluazifop-butyl—The NOAEL and the LOAEL for Fluazifop-butyl in the 1-year dog study should be 25 mg/kg/day and 125 mg/kg/day, respectively, because the decreased cholesterol levels were regarded as marginal at 25 mg/kg/day. Thus NOAELS and LOAELs were comparable in the 13-week and 1-year dog studies.
- Fosetyl-aluminum—The NOAELs are comparable in the 13-week and 1-year studies. There was a difference in target organ toxicity because testicular degeneration was seen in the 1-year dog study but not in the 13-week dog study or in the rodent studies. In the 1-year dog study testicular effects at the LOAEL (20,000 ppm; mid dose) were “minimal” degenerative changes in the testes in both severity and incidence. At the high dose (40,000 ppm), the incidence was higher whereas severity remained minimal. In contrast, in the 13-week study, changes in serum potassium and urea levels were reported at the LOAEL (50,000 ppm; high dose). The serum biochemistry changes were related to the primary mode of action of this chemical, perturbation of the electrolytes balance leading to formation of calculi and irritation of the urinary bladder. In addition, the highest dose tested (50,000 ppm) in the 13-week study was higher than that in the 1-year study (40,000 ppm). However, the dose levels for establishing the NOAEL for 13-week and 1-year dog studies were the same (10,000 ppm). Therefore, if the NOAEL from the 13-week study were to be selected for risk assessment, it would be protective of the testicular effects seen in the 1-year study.
- Mesosulfuron-methyl—The minimal local (gastric) effects seen in the 1-year study were considered to be due to the high treatment dose (574 mg/kg/day) over a prolonged period of time. The overall toxicity profile shows minimal toxicity at doses close to the limit dose.
- Pyrimethanil—Although the 1-year dog study with Pyrimethanil had a lower NOAEL, this is likely an artifact of dose selection and the 13-week dog NOAEL would be protective of the 1-year dog toxicity at the LOAEL. However, Pyrimethanil is known to perturb thyroid homeostasis and lead to hypothyroidism in the rat, which was not identified by either the 13-week or 1-year dog study.
- Spirodiclofen—The extrapolated NOAEL from the developmental neurotoxicity study in the rat (LOAEL: 6.5 mg/kg/day ÷ 10 Uncertainty Factor (UF) = 0.65 mg/kg/day) would be protective of the testicular effects seen in the 1-year dog study. Testicular effects were also identified in the chronic rat study.

In summary, out of the 40 pesticides, differences in the NOAEL and LOAELs of 31 pesticides (depicted in Table 1B) could generally be attributed to dose spacing and selection, experimental variability, or selection of LOAELs based on marginal toxicities observed in the 1-year study. In several cases, reevaluation of the dog data resulted in selection of different NOAELs, LOAELs, and/or critical effects, resulting in essentially the same RfD determination. None of the 31 pesticides with differences in the NOAELs/LOAELs of 1.5-fold or greater between the 13-week and 1-year dog studies would have been under regulated because of these differences.

For 9 pesticides (listed in Table 1C) out of the 40 pesticides, differences in NOAELs and LOAELs could not be ascribed to dose selection. Of these nine pesticides, the chronic RfD for five pesticides (Bifenthrin, Cypermethrin, Bispyribac Hexazinone, and Tebuconazole) were based on results from the 1-year dog study and the chronic RfDs for four were based on results from rodent studies (usually rat chronic study).

Of the five pesticides whose chronic RfDs are based on the 1-year dog study, two were pyrethroids—Bifenthrin and Cypermethrin. The neurotoxic effects observed in the 13-week and 1-year studies were similar except one death was reported at the LOAEL in the 1-year dog study for Cypermethrin, whereas no deaths occurred in the 13-week dog study or in any rat study. The neurotoxic effects of pyrethroids are generally found within 13 weeks and are transient. Because this observation is not consistent with our knowledge about the nature and onset of pyrethroid toxicity, the lower LOAELs for neurotoxic effects in the 1-year study compared to the 13-week study are likely due to experimental variability and dose spacing. For both of these pyrethroids, the available rat data on neurotoxicity (i.e., from the chronic, 2-generation reproductive, or 90-day neurotoxicity studies) would have provided comparable NOAELs and LOAELs.

For Bispyribac, the NOAEL was 10 mg/kg/day and the LOAEL was 100 mg/kg/day based on liver effects in the 1-year dog study. However, the 13-week study's NOAEL of 100 mg/kg/day based on liver effects at 600 mg/kg/day would not be protective. In the absence of the 1-year dog study, however, the critical study for chronic RfD would be the chronic rat study where a NOAEL of 10.9 mg/kg/day and a LOAEL of 194 mg/kg/day was selected based on liver effects. Thus, the target organ and the NOAELs and LOAELs in 1-year dog and chronic rat studies are very similar. Thus, the absence of the 1-year dog study would not have an impact on hazard characterization or RfD derivation.

The toxicity endpoint for Hexazinone in both the 13-week and 1-year dog studies was serum enzyme changes (liver). In the 1-year study, the NOAEL was 5.0 mg/kg/day based on a LOAEL of 37.6 mg/kg/day. The 13-week study's NOAEL of 26 mg/kg/day may not be considered protective. However, the chronic rat study's NOAEL was 10 mg/kg/day based on the LOAEL of 53 mg/kg/day for liver effects. Thus, the chronic rat study that identified a same target organ would have been protective and used for establishing the chronic RfD in the absence of the 1-year dog study.

The toxicity endpoints for Tebuconazole in both the 1-year and 13-week dog studies were similar (adrenal, ocular, and liver effects). In the 1-year study, the NOAEL was 2.94 mg/kg/day and the LOAEL was 4.39 mg/kg/day. The 13-week NOAEL of 7.5 mg/kg/day would not be protective of the LOAEL in the 1-year study. In the absence of the 1-year dog study, however, the chronic rat study NOAEL of 5.3 mg/kg/day would be used for the risk assessment. It is unclear whether the differences in NOAELs between the two dog studies are significant. It should be noted, however, that because of the US EPA 2006 retrospective dog study analysis, a rat neurodevelopmental study was selected for the basis of the chronic RfD for this chemical and is considered protective of all populations including infants and children (see http://www.epa.gov/EPA-PEST/2009/March/Day-04/p4373.htm).

The other four pesticides in which the 1-year dog study provided a lower NOAEL and /or LOAEL included Fluazinam, Mancozeb, Thiamthoxam, and Thiram. The absence of the 1-year dog study for these pesticides would not have an impact on the risk assessment because the rodent (typically rat) was the most sensitive species and the basis for the chronic RfD.

Discussion

EPA has historically required testing in both a rodent and a nonrodent species. It is common practice for the nonrodent species to be the dog. There has been much debate regarding whether it is appropriate to use the dog as a second species in regulatory testing, and the value of information gained (Appelman and Feron, 1986; Lumley et al., 1992; Parkinson et al., 1995; Gerbracht and Spielmann, 1998; DeGeorge et al., 1999; Spielmann and Gerbracht, 2001; Baetcke et al., 2005; Box and Spielmann, 2005).

EPA uses test data to determine what levels of environmental exposures are acceptable. In comparisons of results from shorter-term to longer-duration dog toxicity studies, significant information is rarely gained from a longer-term dog study (>13 weeks). Lumley et al. (1992) evaluated the minimum duration of chronic animal toxicity studies needed to detect adverse responses and to define safety margins between the proposed use levels and adverse responses for pharmaceutical compounds. In the results of toxicity studies conducted with dogs, all significant effects were identified within 6 months for 98% of the pharmaceuticals (55 of 56 case studies). Parkinson et al. (1995) analyzed 117 pharmaceuticals in the UK Centre for Medicines Research toxicology database and determined that dog studies >6 months demonstrated additional effects in only 13 of the 117 (11%) compounds. For most of the chemicals, the significant effects were seen within 3 months. In cases where additional toxicities were identified after 3 months, similar responses were seen in the rat studies. These authors suggested that dog studies longer than 3 months provide relatively little new toxicological information.

Spielmann and Gerbracht (2001) (also reported in Box and Spielmann, 2005) performed a comprehensive analysis of data submitted to the Federal Institute of Health Protection of Consumers and Veterinary Medicine (Germany) from dog studies on pesticides to determine whether chronic dog studies provided important additional information not provided by studies of shorter duration. They reported that "analysis of the severity of organ-specific toxic effects of pesticides revealed that chronic long-term studies (52/104 weeks) in dogs do not provide specific additional information to 26-week studies in the same species." They further stated that "safety testing of pesticides in dogs should be limited to subchronic (13-week) studies since an extension of the duration of the studies does not provide additional essential information." The recommendation for a study of 13-weeks duration was supported by the finding that in only 5% was new and relevant information on the toxic properties of the pesticides provided by chronic dog studies that was not seen in dog studies of shorter duration or in studies with rats or mice.

In this analysis, there are only 9 of the 110 pesticides (8%) where there are indications that a 1-year dog toxicity study could potentially provide a lower LOAEL than a 13-week study for purposes of RfD derivation. These cases were random across different pesticide classes and, overall, there was no regulatory impact due to the absence of a 1-year dog study because available rodent toxicity studies provided comparable NOAELs and LOAELs.

On rare occasions, significant new toxicities were identified in the 1-year dog studies that were not observed in the 13-week study or in the rodent studies. One case involves Cypermethrin where a death occurred in the 1-year dog study that was not observed in the 13-week dog and rodent studies. This observation is unexplainable and would not be expected given that the neurotoxic effects of pyrethroids are generally found within 13 weeks and are transient. Furthermore, these compounds typically reach kinetic steady state within 1–6 days depending on the chemical and do not bioaccumulate. Another situation was Fosetyl-Al where testicular degeneration was found at 500 mg/kg bw in the chronic dog study but was not reported in the rodent or 13-week dog study. The primary mode of action for Fosetyl-Al is disruption in urinary physiology, including precipitation of calcium and phosphorus and formation of calculi, which in turn irritate the urothelium of the bladder, resulting in toxicity, hyperplasia, and bladder tumors; the latter effects were better characterized in rat studies. Nonetheless, the 13-week dog NOAEL would have been protective of the testicular lesions seen at the chronic LOAEL in the chronic dog studies.

The conclusion from this evaluation of 110 pesticide chemicals is similar to that reached by Spielmann and Gerbracht (2001), Box and Spielmann (2005), and Doe et al. (2006). Extension of a dog toxicity study beyond 13 weeks does not provide additional, essential information. Data from the chronic rodent and 13-week dog studies would provide an adequate basis for RfD derivation and assessing risks from exposure to pesticide chemicals. Missing a critical toxic effect in the absence of the 1-year dog study is considered to be of low probablity given that the rat and 13-week dog study generally identified similar effects and comparable NOAEL/LOAELs.

Conclusion

Longer-duration studies (e.g., 1 year) in the dog do not result in appreciably lower NOAELs or identify new toxic effects for the majority of chemicals when compared to the shorter-duration 13-week study in this species. This conclusion is consistent with the analysis of 141 pesticides (for which 12-month study is considered relevant) sponsored by the German Federal Institute for Risk Assessment (now Federal Institute for Risk assessment; Spielmann and Gerbracht, 2001; Box and Spielmann,2005) where it was concluded that 1-year (or 2-year) dog studies are of limited value because they provide "essential" information in only a few cases.

The findings of the German and US retrospective analyses of pesticide dog toxicity results yielded remarkably similar conclusions. Even for the 8% where there are indications that a 1-year dog toxicity study would potentially provide a lower LOAEL than a 13-week study for purposes of RfD derivation, differences between LOAELs and NOAELS between the two dog studies were small (4-fold or less). In no case did these small differences have a regulatory impact on pesticide risk assessments; data from the required chronic rodent studies, 2-generation rat reproduction study, and the 13-week dog toxicity study provided an adequate basis for chronic RfD derivation for pesticide risk assessment. As a result of this analysis, the US EPA Office of Pesticide Programs eliminated the routine requirement of the 1-year dog study while retaining the requirement for the13-week dog study (US EPA, 2007).

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Declaration of interest

All of the authors are employees of the US EPA, Office of Pesticide Programs. The authors carried out this work as a normal part of their official government duties. This work summarizes a more extensive analysis that was used in support of decisions regarding dog toxicity testing studies by the US EPA in updating its data requirements in part 158 of Title 40 in the Code of Federal Regulations for the registration

of conventional pesticide products. The authors have sole responsibility for the writing and content of the paper, which may not necessarily reflect the views of the US EPA.

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